

The University of Chicago

FORMATIVE EFFECTS OF CERTAIN  
SUBSTITUTED CHLOROPHENOXY  
COMPOUNDS ON BEAN LEAVES

A DISSERTATION SUBMITTED TO THE FACULTY OF  
THE DIVISION OF THE BIOLOGICAL SCIENCES IN  
CANDIDACY FOR THE DEGREE OF DOCTOR OF  
PHILOSOPHY

DEPARTMENT OF BOTANY  
1947

By  
DANIEL F. BURTON

Reprinted from  
THE BOTANICAL GAZETTE  
Vol. 109, No. 2, December, 1947





The University of Chicago

FORMATIVE EFFECTS OF CERTAIN  
SUBSTITUTED CHLOROPHENOXY  
COMPOUNDS ON BEAN LEAVES

A DISSERTATION SUBMITTED TO THE FACULTY OF  
THE DIVISION OF THE BIOLOGICAL SCIENCES IN  
CANDIDACY FOR THE DEGREE OF DOCTOR OF  
PHILOSOPHY

DEPARTMENT OF BOTANY

1947

By

DANIEL F. BURTON

Reprinted from  
THE BOTANICAL GAZETTE  
Vol. 109, No. 2, December, 1947





## FORMATIVE EFFECTS OF CERTAIN SUBSTITUTED CHLORO-PHENOXY COMPOUNDS ON BEAN LEAVES<sup>1</sup>

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 594

DANIEL F. BURTON

### Introduction

One of the most striking effects of the application of substituted phenoxy compounds to plants is the induction of leaf modifications. ZIMMERMAN (10, 11) has stressed the importance of the study of modifications in form induced by the hormone-like substituted phenoxy compounds by pointing out that protoplasm is capable of a variety of reactions and that it is desirable to investigate any

mechanism that determines which of the potential reactions actually shall occur. He also suggested that, since form is determined by cell behavior, the substituted phenoxy compounds and the substances secreted by the nucleus may control cytoplasmic activity in a similar fashion and hence control form.

FOSTER (5) has indicated the paucity of data on leaf histogenesis compared with analogous data on stems and roots. Therefore, in the present experiments the normal growth pattern in leaves of the Red Kidney bean was examined as well

<sup>1</sup> This work was supported in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.



as the growth patterns induced by the application of growth-regulating substances.

### Material and methods

Red Kidney beans were planted in flats containing fertile loam soil and placed on greenhouse benches for germination. When the primary heart-shaped leaves were approaching their mature size and the first trifoliate leaves were starting to emerge from the bud, the flats were divided into four groups. The plants in the first group were treated with 0.5% 2-chlorophenoxyacetic acid in Carbowax 1500 applied to the upper surface of the blade near the bases of the primary leaves, in amounts estimated to contain 40 mg. of the mixture per plant, or 200  $\gamma$  of the acid (3). The second, third, and fourth groups were treated with 4-chlorophenoxyacetic acid in lanolin, 2,4-dichlorophenoxyacetic acid (2,4-D) in lanolin, and 2,4-dichlorophenoxyacetic acid in Carbowax, respectively, in the same concentrations and in amounts approximating those used in the first group. Some plants in each flat were left untreated as controls.

Collections of buds, young leaves, and fully developed leaves were made from treated plants and controls. The material was fixed in Navashin's solution, handled according to the tertiary butyl-alcohol method, sectioned at 10–15  $\mu$ , and stained with KRAUS's modification of Fleming's triple stain.

Three sets of beans were planted at Chicago. The first set was planted on March 27, 1946, treated on April 6, and leaves and buds were collected on May 9. The second set was planted on April 11 and treated on April 22. At the time of treatment of this set, temperatures in the greenhouse were unseasonably high, and so many of the plants died that no

collections were made. A third set was planted on May 21, treated on June 1, and collections were made on June 22. A fourth set, planted at State College, Mississippi, on March 26, 1947, was treated only with 2,4-D in lanolin and in Carbowax, on April 8; collections were made on April 29.

### Observations

DEVELOPMENT AND APPEARANCE OF NORMAL TRIFOLIATE LEAVES.—The internal tissues of the lamina of a normal leaflet originate by the activity of sub-epidermal marginal meristems which are found on the lateral edges of the embryonic midrib nearer the adaxial surface (fig. 2A). Divisions of cells of these meristems give rise to four layers of cells between the upper and lower epidermis (fig. 2B). These layers plus the upper and lower epidermis constitute the plate meristem. The cells of the adaxial layer of internal tissue are larger than those of the other internal layers. Since the six cell layers in the lamina of the embryonic leaflet are still present in the lamina of a leaflet just prior to the development of intercellular spaces, all division of cells in the plate meristem, except divisions involving veins, must produce daughter cells in the plane of the plate meristem.

Initial cell divisions in the development of veins of the minor vascular network produce daughter cells vertical to the plane of the plate meristem. Such divisions occur in a single row of cells in the layer immediately beneath the layer of enlarged cells. In cross section, this row appears as a single cell. Figure 2C, illustrating a section cut obliquely to the row, shows a cell in which one such division has occurred, giving rise to the first two cells of the veinlet. To the right of these cells there were two divisions, producing three cells. Figure 2D shows a later stage





FIG. 1.—Trifoliate leaves from control (*A*) and treated plants (*B–H*). Plants treated with (*B*) 2,4-D in Carbowax 1500; reduced size and increased prominence of veins compared with control; (*C*) 2,4-D in lanolin; effects similar to *B*; (*D*) 2-chlorophenoxyacetic acid; leaflets reduced and prominent veins similar to *C*; (*E*) 4-chlorophenoxyacetic acid; narrow straplike leaflets ultimately produced; (*F, G, H*), 2,4-D in lanolin; leaves taken in succession to illustrate limitation in size of later leaves.



in the formation of smaller veins. An adjacent cell of the same layer has divided, as have several cells in the next lower layer.

A section through the same leaflet as that shown in figures 2*A* and 2*B* is illustrated in figure 2*E*. Although the tissues at the level of figures 2*A* and 2*B* were embryonic, those at the tip of the leaflet were mature, thus demonstrating that

maturation begins at the tip. When fully expanded, the leaflet has a single layer of palisade parenchyma beneath the upper epidermis (fig. 3*A*), derived from the upper internal layer of enlarged cells illustrated in figures 2*A*, *B*, *C*, and *D*. The spongy mesophyll is derived from the other three internal layers (fig. 3*A*).

The vascular elements of the midrib (fig. 3*A*) and the largest veins are sur-

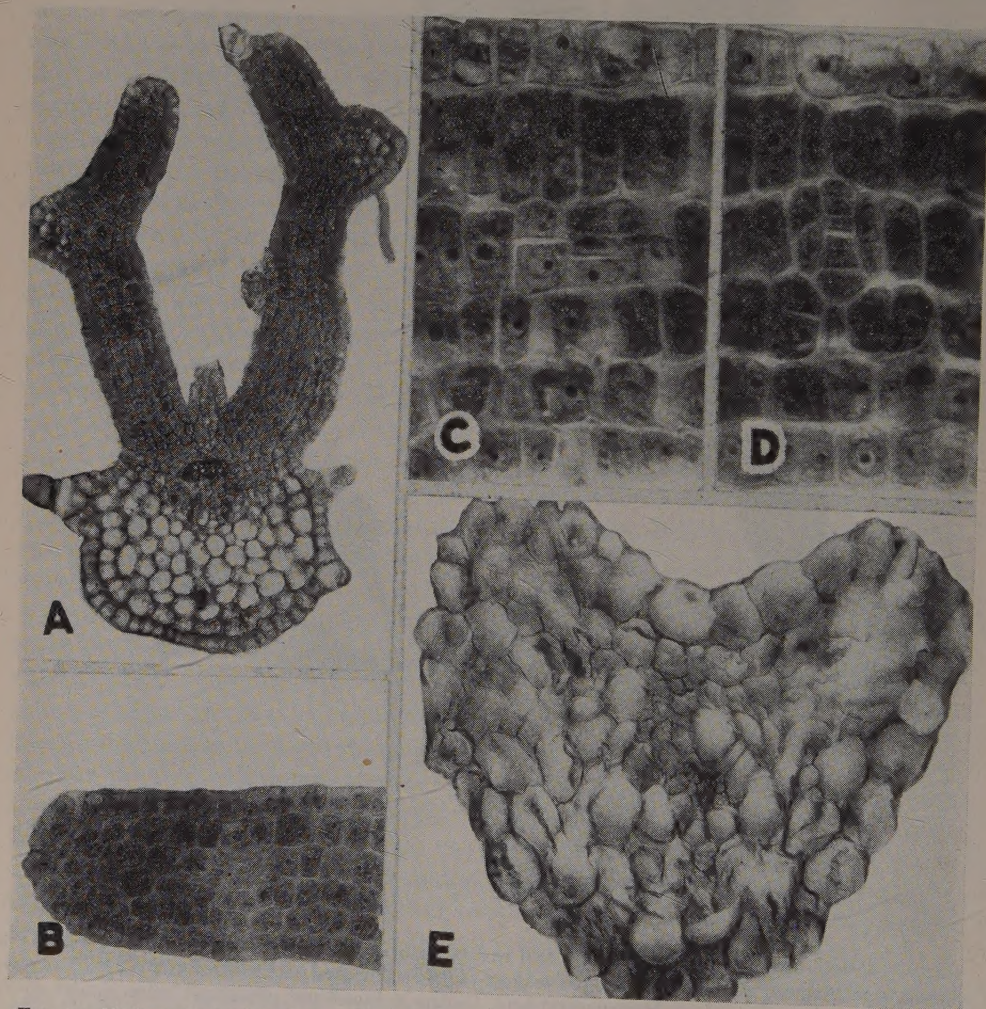


FIG. 2.—Embryonic leaflet of control. *A*, cross section through leaflet; lamina arising from embryonic midrib. *B*, detail of marginal and plate meristems shown in *A*. *C*, oblique section through row of cells initiating a vein in upper layer of spongy-mesophyll plate-meristem. *D*, cross section through similar row of cells, illustrating more advanced stage of vein formation. *E*, tip of leaflet, showing maturity of tissues at this region of same leaflet shown in *A* and *B*.



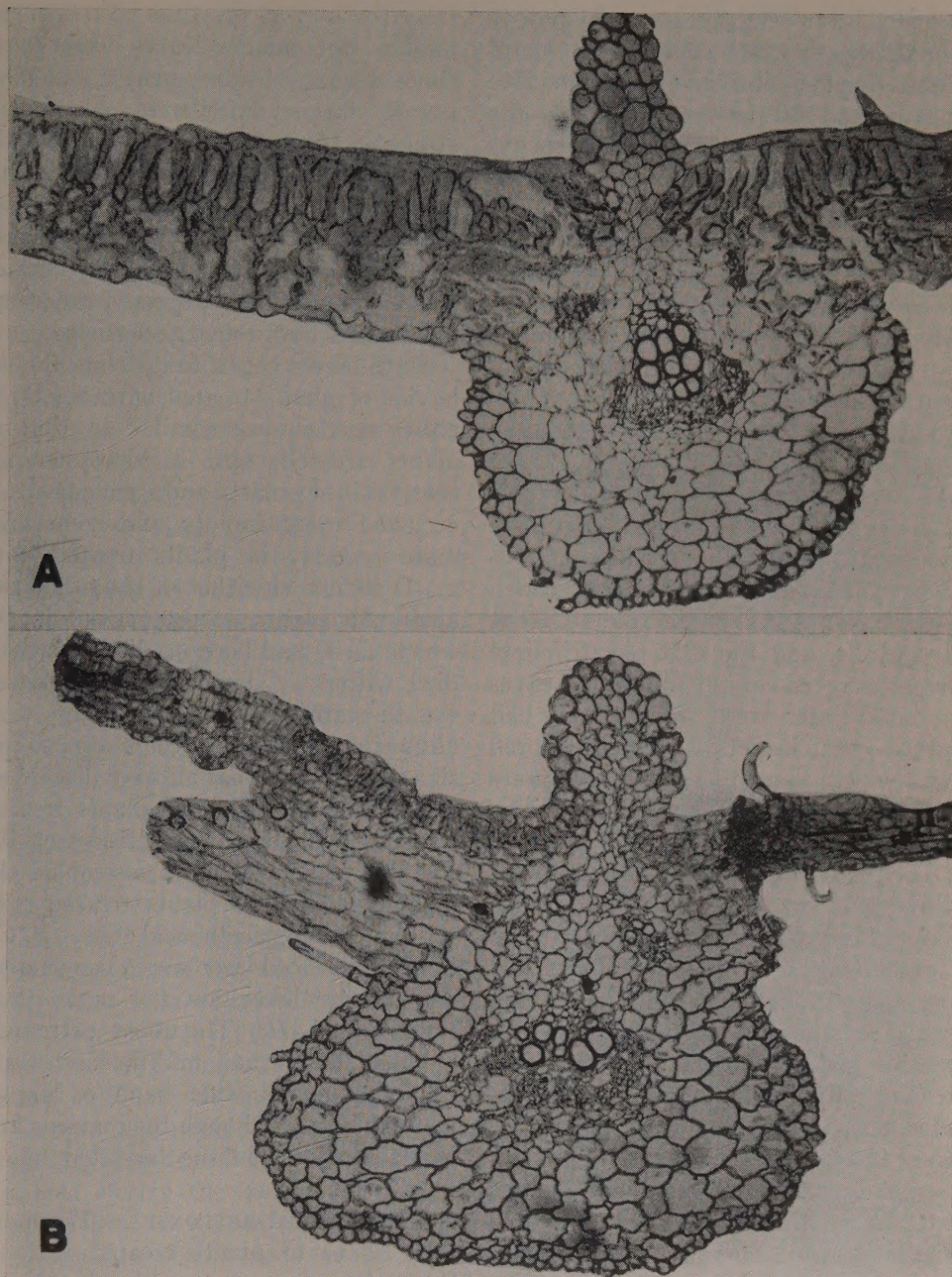


FIG. 3.—Cross section through mature leaflet in region of midrib of: (A) control with well-developed intercellular spaces in mesophyll and extensive region of parenchyma around vascular elements; (B) plant treated with 2-chlorophenoxyacetic acid; normal vascular tissue but no intercellular spaces in mesophyll.



rounded by an extensive parenchymatous bundle sheath which contains no chloroplasts. Smaller veins have a less extensive sheath, and in the smallest veins the sheath is only a single layer of parenchymatous cells often containing chloroplasts.

The mature leaf is trifoliate. The lamina of a leaflet is marked by a network of fine anastomosing veins (fig. 1A). A stipule subtends each of the lateral leaflets, with an additional pair beneath the terminal leaflet. Stipules persisted in the normal position in treated plants.

GROSS EFFECTS OF TREATMENTS.—Little or no epinasty or stem curvature occurred in plants treated with 2-chlorophenoxyacetic acid. At first the growth of these plants was comparable with that of controls, and the first few trifoliate leaves were essentially normal. Leaves produced later were smaller and had darker green leaflets with light-colored veins which, though less numerous, were more prominent than the veins of controls (fig. 1D).

The plants to which 4-chlorophenoxyacetic acid was applied showed pronounced epinasty and stem curvature in a few hours. Curvature frequently turned the stem through  $180^\circ$ , so that it appeared to have reversed from negative to positive geotropism. In the second lot, treated during a period of high temperatures, 80% of the plants were dead in 17 days. If a plant was not killed, its tip eventually resumed an upright growth pattern; by this return to the normal orientation an S-curve was produced in the hypocotyl and first internode. The first trifoliate leaf produced on a partly recovered stem was similar in appearance to the modified leaves produced on plants treated with 2-chlorophenoxyacetic acid. This leaf was just starting to emerge

from the bud at the time of treatment. Similar but smaller leaves were sometimes developed subsequently, but most leaves formed later were narrow and straplike (fig. 1E). In such leaves the unpigmented midrib was wider than that of normal leaflets and often composed four-fifths of the total width of such anomalous structures. No other venation was apparent. The margins of the straplike leaflets were rolled and green.

Until leaves began to develop, the behavior of plants treated with 2,4-D, in either carrier, was similar to that of plants treated with 4-chlorophenoxyacetic acid. Epinasty and stem curvature occurred more rapidly and sometimes more severely in plants treated with 2,4-D than with either of the other two acids. All plants in the second lot to which 2,4-D had been applied in lanolin died within 17 days. When Carbowax was the carrier, most of the plants were alive after 17 days, but there were so few signs of recovery that all were discarded.

In those lots in which plants treated with 2,4-D showed partial recovery, the first few leaves produced resembled the modified leaves on plants treated with 2-chlorophenoxyacetic acid (figs. 1B, C). Leaves produced later were even smaller than earlier leaves on the same plant (figs. 1F, G, H). The most extremely modified leaves had midribs and veins fasciated into a wide band of unpigmented tissue, although the margins and occasional islets in the fasciated bands were green.

HISTOLOGICAL RESPONSES.—The modified leaves of plants treated with 2-chlorophenoxyacetic acid developed much like those of controls until the stage at which intercellular spaces normally would have appeared. Expansion of chlorenchymatous layers then ceased, and no intercellular spaces were formed



in either the palisade or spongy mesophyll (fig. 4A). Since the cells of these tissues contained chloroplasts, there was a greater amount of green pigment in a given volume of the leaflet than in a like volume of a normal leaflet containing intercellular spaces. There was no histological evidence of changes in the midrib or major veins (fig. 3B), so that the increased prominence of these veins in gross aspect was probably a result of contrast with the darker green mesophyll. The darker color of the latter probably helped to obscure the minor veins, since they were not surrounded by an unpigmented parenchyma extending from the lower to the upper epidermis.

Laminae of leaflets from plants treated with 2,4-D developed internal structures by the activity of marginal meristems similar to the marginal meristems of controls (fig. 5A). These meristems laid down four internal layers of plate meristem (fig. 5B) similar to the corresponding layers in the controls. Thus, the six layers of plate meristem described for the embryonic leaves of controls were present in the embryonic leaves of treated plants.

As in the controls, maturation of tissues began at the tip of a leaflet. Figure 4B shows a cross section through the mature tissues at the tip of the same leaflet which had embryonic tissue at the level shown in figures 5A and 5B. The tissues of mature laminae of the earliest-formed leaflets of plants treated with 2,4-D resembled closely the comparable tissues from leaflets of plants treated with 2-chlorophenoxyacetic acid. In the later-formed leaves of plants treated with 2,4-D, fasciation of the veins was extensive. The vascular elements were discrete, but the unpigmented parenchyma surrounding the vascular elements of the midrib and major veins was continuous

through much of the width of the leaflet. Chlorenchymatous tissue was confined to the margins and occasional islets surrounded by fasciated veins. There were no intercellular spaces in the chlorenchyma. Figure 5C shows a cross section from an extreme example of the smaller leaflets.

In the narrow, straplike leaflets of plants treated with 4-chlorophenoxyacetic acid, fasciation of the veins was

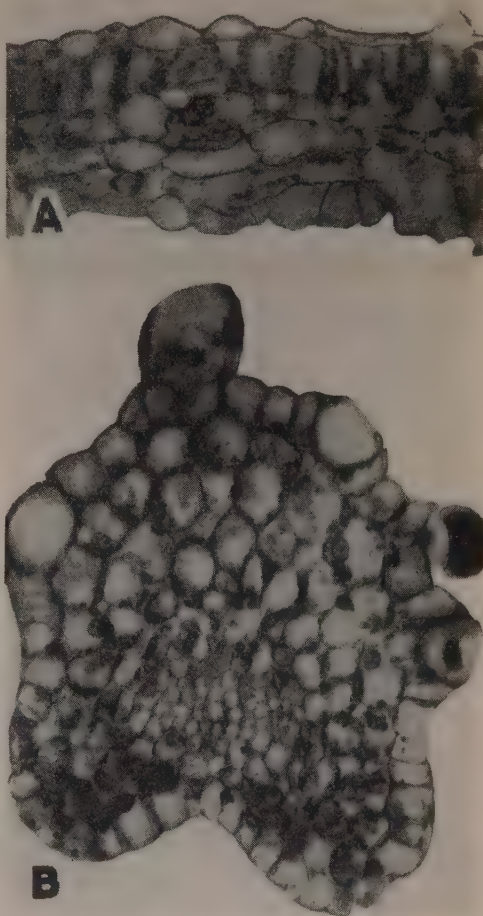


FIG. 4.—Section through (A) lamina of leaflet of plant treated with 2-chlorophenoxyacetic acid, illustrating failure to develop intercellular spaces; (B) tip of embryonic leaflet from plant treated with 2,4-D, illustrating maturity of tissue.



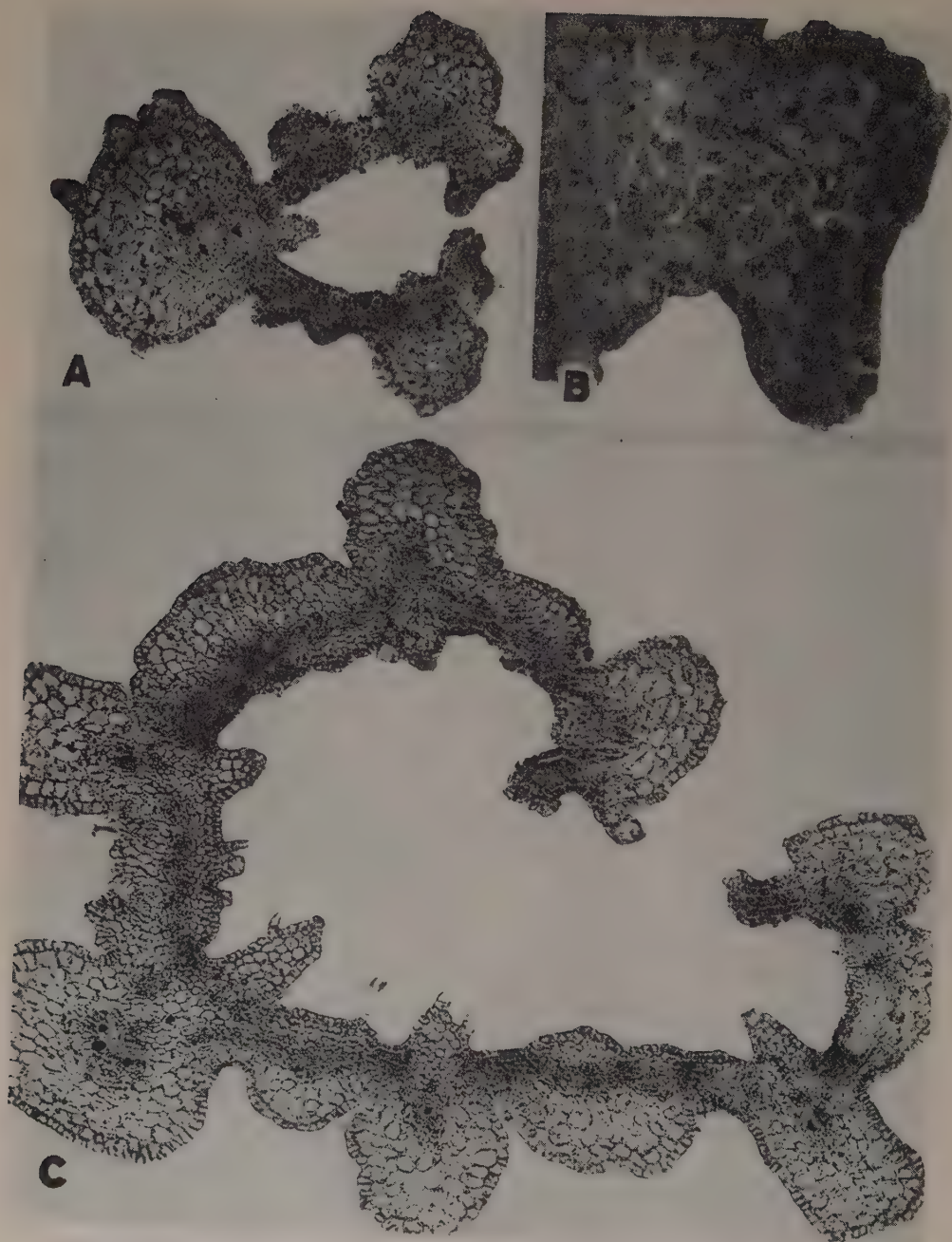


FIG. 5.—Cross sections of embryonic leaflet shown in fig. 4*B*, showing (*A*) origin of lamina and (*B*) details of marginal and plate meristems. *C*, cross section through extremely modified mature leaflet of plant treated with 2,4-D, showing fasciation of veins and chlorenchymatous tissue as dark-staining regions at margin and in islets between veins.



complete (fig. 6). Parenchyma surrounding the midrib and major veins was continuous from the upper to the lower epidermis. The parenchyma cells of the major veins contained no chlorophyll so that chlorenchymatous tissue was confined to a narrow margin of the leaflet. The vascular elements remained discrete and appeared as separate bundles imbedded in the parenchyma. Parenchyma cells farthest from the vascular elements were largest and those adjacent to the vascular elements were smallest. The vascular elements and the small parenchyma cells surrounding them formed a

plate within the larger plate of parenchyma. At the extreme margin were a few chlorenchymatous cells that usually were confined to the layer corresponding to palisade in normal leaves.

### Discussion

The gross responses observed in treated bean plants were similar to those previously reported. SWANSON (9) sprayed Red Kidney bean plants with 2,4-D and noticed epinasty and stem curvature in a few hours. The plants he observed also produced tiny leaves. BEAL (2, 3, 4) treated sweet pea, African mari-

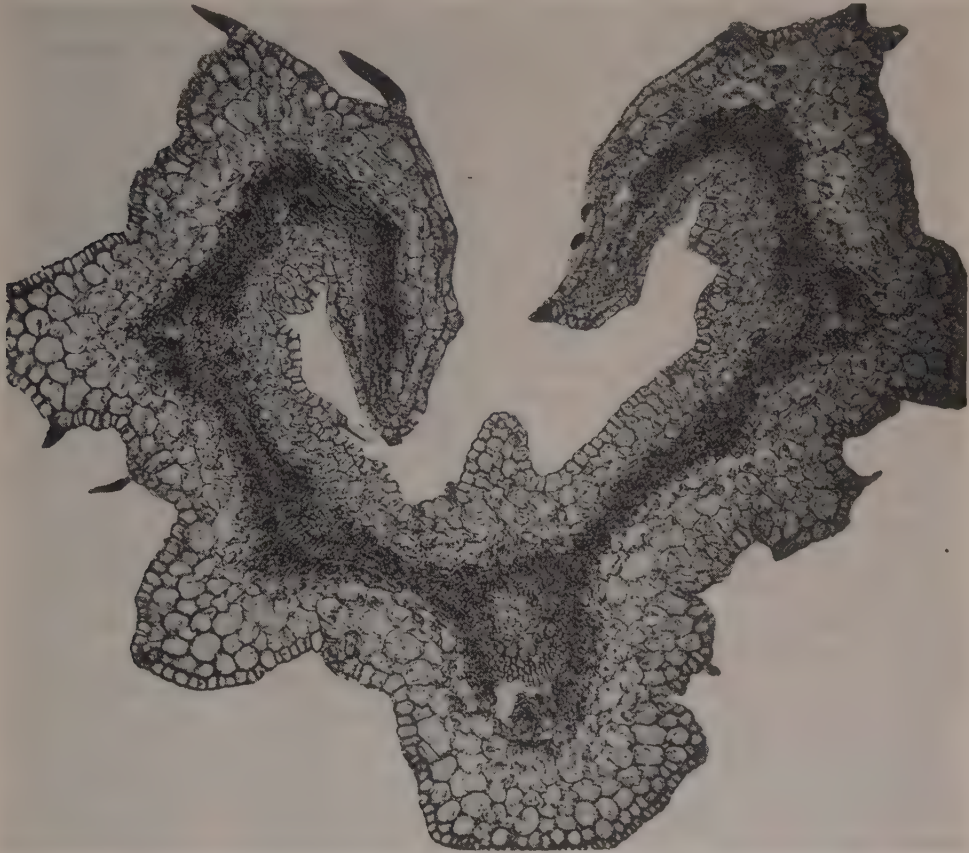


FIG. 6.—Cross section through narrow, rolled leaflet ultimately produced by plants treated with 4-chlorophenoxyacetic acid. Veins are fasciated, and vascular elements and small parenchyma cells surrounding them appear as darkly stained region across larger parenchyma region. Chlorenchyma confined to margin.



gold, and Red Kidney bean plants with substituted chlorophenoxy and other compounds. His experiments demonstrated that responses to treatment with 2-chlorophenoxyacetic acid applied in Carbowax were greater than when applied in lanolin. Conversely, 4-chlorophenoxyacetic acid applied in lanolin produced a marked response, although the same compound applied in Carbowax was ineffective. As in the present experiments, responses to 2,4-D were nearly indifferent to the carrier used.

MARTH and DAVIS (6) showed that temperature is of significance in the induction of responses to 2,4-D. If temperatures were too high, killing commonly resulted. In the present experiments concentrations of 0.5% of acid in the carrier were found to induce changes in the growth pattern at ordinary spring temperatures in a greenhouse. This concentration of 2,4-D and of 4-chlorophenoxyacetic acid was lethal at higher temperatures.

ZIMMERMAN and HITCHCOCK (12) reported formative effects of several phenoxy compounds on tobacco, tomato, and other plants. Some of their figures indicate progressive modification of leaves of plants treated with 4-chlorophenoxyacetic acid. The comparative stage of development of these leaves at the time of treatment is not clear. In the present study and in the experiments of BEAL (3), if the first trifoliate leaf was starting to emerge from the bud at the time of treatment with 4-chlorophenoxyacetic acid, its mature leaflets resembled the modified leaflets of plants treated with 2-chlorophenoxyacetic acid. Second and third trifoliate leaves, although usually similar in appearance, were much smaller. Subsequent leaves were almost uniform in size and had a narrow straplike form

(fig. 1E). These later leaves were present only as primordia or perhaps were not yet differentiated at the time of treatment. This suggests that, if leaves are young enough, a uniform response to treatment with 4-chlorophenoxyacetic acid might occur. There was a suggestion of eventual uniformity of response in ZIMMERMAN and HITCHCOCK's report of successive whorls of fasciated leaves of tobacco treated with 4-chlorophenoxyacetic acid.

Modification in response to applications of 2,4-D produced a gradation in size that extended to leaves not yet apparent in the buds at the time of treatment. On the basis of the present experiments, it could not be determined whether the reduction in size of leaves developed following the application of 2-chlorophenoxyacetic acid was similarly progressive or whether the size gradation was a consequence of the relative immaturity of the later-formed leaves at the end of the experiments.

The development of the lamina of the untreated Red Kidney bean leaflet is in many respects similar to that observed in tobacco by AVERY (1). The subepidermal, marginal meristem in both plants gives rise to four layers within the epidermis. These layers remain four in the bean but become five in tobacco by division of one of the two inner layers. In both plants the adaxial of these four internal layers become palisade parenchyma, while the other three internal layers (four in tobacco) differentiate into spongy mesophyll. Subsequent to the formation of the four layers of plate meristems in bean leaflets and the five layers in tobacco leaves, all cell divisions produced new cells in the plane of the plate meristems, except those involving vein formation. The divisions in this

plane led SCHUEPP (7) to term these layers "Plattenmeristem." SMITH (8) showed that the constancy in the number of layers, and hence extension of the lamina by growth of plate meristem, is of regular occurrence in embryonic leaves with the exception of certain sun leaves.

Veins of the minor vascular network in bean leaflets originate in the second layer beneath the adaxial epidermis. AVERY (1) stated that this is the usual mode of origin in tobacco. FOSTER (5), in his summary, showed diagrams indicating that veins usually originate in the layer immediately beneath the embryonic palisade or palisades.

According to AVERY (1), the development of intercellular spaces in mesophyll is a result of continued cell enlargement in the epidermis after cell division and enlargement in the mesophyll have ceased. The compounds used in the present experiments inhibited the formation of intercellular spaces. This possibly indicated that the epidermal cells of the laminae of bean plants treated with substituted phenoxy compounds failed to enlarge. There is also the possibility that lysis of the middle lamellae of mesophyll cells failed to occur. This would render epidermal cell enlargement ineffective in producing intercellular spaces. Since the mesophyll cells then would not separate, the epidermal cells would be forced to increase their volume by periclinal expansion.

The narrow leaflets of plants treated with 4-chlorophenoxyacetic acid indicated suppression of the activity of the plate meristem. The resulting approximation of veins resulted in fasciation. The parenchyma surrounding the veins became continuous although the vascular elements remained discrete. The laminae of leaflets from plants treated with 2,4-D

exhibited an internal structure in the earlier leaves similar to that induced by 2-chlorophenoxyacetic acid. The gradation from this form to leaflets resembling those from plants treated with 4-chlorophenoxyacetic acid has been noted. This indicated a progressive inhibition of cell division in the plate meristem, for the laminal meristem was present and functional.

### Summary

1. A bean leaflet develops a lamina by the activity of a subepidermal marginal meristem, which produces four internal layers of plate meristem. The adaxial of these layers develops into the palisade layer; the other three produce the spongy mesophyll.

2. Veins of the minor network are initiated by divisions of a row of cells in the layer beneath the embryonic palisade. Additional cells and additional layers become involved.

3. Treatment of young plants with 0.5% concentration of 2-chlorophenoxyacetic acid in Carbowax inhibited the formation of intercellular spaces in the mesophyll of bean leaflets.

4. Similar application of 0.5% 4-chlorophenoxyacetic acid in lanolin inhibited the activity of plate meristem in the laminae of bean leaflets. As a result, veins were approximate and their parenchyma became continuous although the vascular elements remained discrete. Chlorenchymatous tissue was confined to the margin.

5. Similar treatment with 0.5% concentrations of 2,4-dichlorophenoxyacetic acid, in either lanolin or Carbowax as the carrier, induced progressive modification of bean leaves. The earliest leaves were similar to those induced by treatment with 2-chlorophenoxyacetic acid. In later



leaves the external form and internal structure resembled that found in leaflets of plants treated with 4-chlorophenoxyacetic acid.

The writer wishes to express his appreciation of the advice and encouragement

tendered by Dr. JOHN M. BEAL and other members of the Department of Botany of the University of Chicago in the course of this work.

DEPARTMENT OF BOTANY  
MISSISSIPPI STATE COLLEGE  
STATE COLLEGE, MISSISSIPPI

#### LITERATURE CITED

1. AVERY, G. S., JR. Structure and development of the tobacco leaf. *Amer. Jour. Bot.* **20**:565-592. 1933.
2. BEAL, J. M. Some teleomorphic effects induced in sweet pea by application of 4-chlorophenoxyacetic acid. *BOT. GAZ.* **105**:471-474. 1944.
3. ———. Further observations on the teleomorphic effects of certain growth-regulating substances. *BOT. GAZ.* **106**:165-178. 1944.
4. ———. Histological reactions of bean plants to certain of the substituted phenoxy compounds. *BOT. GAZ.* **107**:200-217. 1945.
5. FOSTER, A. S. Leaf differentiation in angiosperms. *Bot. Rev.* **2**:349-372. 1936.
6. MARTH, P. C., and DAVIS, F. F. Relation of temperature to the selective herbicidal effects of 2,4-dichlorophenoxyacetic acid. *BOT. GAZ.* **106**:463-472. 1945.
7. SCHUEPP, OTTO. Meristeme. *Linsbauer's Handb. der Pflanzenanatomie* **4**. 1926.
8. SMITH, G. H. Anatomy of the embryonic leaf. *Amer. Jour. Bot.* **21**:194-209. 1936.
9. SWANSON, C. P. Histological response of Red Kidney bean to aqueous sprays of 2,4-dichlorophenoxyacetic acid. *BOT. GAZ.* **107**:522-531. 1946.
10. ZIMMERMAN, P. W. Formative influences of growth substances on plants. *Cold Spring Harbor Symposia on Quant. Biol.* **10**:152-157. 1942.
11. ———. The formative influences and comparative effectiveness of various plant hormone-like substances. *Torreya* **43**: 98-115. 1943.
12. ZIMMERMAN, P. W., and HITCHCOCK, A. E. Substituted phenoxy and benzoic acid growth substances and the relation of growth to physiological activity. *Contrib. Boyce Thompson Inst.* **12**:321-343. 1942.











